

Stem Cells To Cure Sickle Cell Anemia

Tolulope O. Rosanwo¹⁻³

¹Division of Pediatric Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Boston, MA, USA

²Stem Cell Transplantation Program, Boston Children's Hospital

³Case Western Reserve University School of Medicine, Cleveland, OH, USA

Correspondence should be addressed to T.R. (tolulope.rosanwo@case.edu)

Sickle cell anemia (SCA) is one of the most prevalent genetic disorders in the world and the most common congenital anemia in the United States. The severity of SCA spans from mild clinical cases, managed by careful living, to severe cases involving bone crises, infection, stroke, and multi-organ failure. Hematopoietic stem cell transplantation (HSCT) is the most widely known and effective method to cure the disease and is used today in only severe cases due to risks of graft rejection and fatal infections. The use of stem cells for the cure of sickle cell disease from the early days of transplantation to the most recent breakthroughs in stem cell therapy, gene therapy and genome-editing will be examined in this review.

Introduction

In 1904, Grenadian dental student, Walter Clement Noel, was admitted to Chicago Presbyterian Hospital with complaints of fatigue and bodily pain. He was first examined by a cardiology resident by the name of Ernest Irons who contacted his attending physician, James B. Herrick when he inspected Noel's cells via blood smear¹. The medical case of the Grenadian with "pear-shaped and elongated" cells and bone pain was published and similar reports were shared from physicians practicing in other states until the strange disease was named "sickle cell anemia" by Vernon Mason in 1922.

Two decades passed until Linus Pauling and Harvey Itano first described sickle cell anemia (SCA) as a "molecular disease." They hypothesized that the distorted shape of sickle cells arose from defects in the hemoglobin structure. Electrophoresis, then a new innovation, proved that the hemoglobin protein from sickle cell patients differed in both size and

charge from healthy patients. This finding, Pauling believed, transformed medicine into a "real science." Disordered protein could cause human disease.

Since the early 20th century, many scientists including J.B.S. Haldane and A.C. Allison made observations that regions with high incidence of malaria also had peoples with blood disorders like the thalassemias and SCA. Over time, it has been postulated that heterozygous carriers of a mutated SCA gene better survive and resist fatal cases of malaria^{2,3}. Today, it is well known that a missense mutation in the beta-globin gene locus found on the 11th chromosome promotes the aberrant generation of the amino acid valine instead of glutamic acid⁴. This amino acid change gives rise to the production of sickle hemoglobin—the key underpinning of sickle cell anemia. Particularly in low oxygen environments, this altered biochemistry causes the hemoglobin molecules to intracellularly polymerize, distorting the cell's shape. Stiff, and elongated, these red blood cells (RBCs)

occlude and damage the body's vasculature. The natural history of SCA often includes the failure of multiple organ systems –especially respiratory failure secondary to acute chest syndrome (ACS), cerebrovascular incidents and sepsis. Millions live with SCA most commonly in malaria-endemic regions in west and central Africa, eastern Saudi Arabia and central India with sickle cell trait reaching a prevalence of 40% in some areas^{5,6}. 1,000 babies are born each day in Africa with SCA and half will die before five years of age mostly from infectious causes⁷.

In the United States during the 1970s, the average person with SCA lived until about 14 years of age⁸. Today, comprehensive care and improved management have pushed the life expectancy to 50-60 years of age in the United States and Great Britain with over 90% of children living into adulthood⁹. Over the course of the average lifespan of a patient with SCA living to 45 years, \$1 million will be incurred in hospital fees as admissions and complications are frequent¹⁰.

With such a personal, financial and global burden of disease, a safe, universal cure for SCA is paramount. To date, stem cell therapy is the only source of a cure for SCA in select patients. While hematopoietic stem cell transplantation (HSCT) is the only means to cure SCA, it is far from universal. This review will focus on the historical, current and future uses of stem cells for the cure of SCA.

The Birth of Hematopoietic Stem Cell Transplantation

The world was unofficially introduced to hematopoietic or “blood-forming” stem cell transplantation following World War II. Ten years after the end of US involvement in WWII, the civilian populations of Nagasaki and Hiroshima, Japan were exposed to large amounts of radiation and were dying of hematopoietic failure and leukemia. Richmond Main and Joan Prehn discovered that radiation syndrome similarly found in mouse models could be prevented by using lead to protect the spleen or injecting it with cells from the bone marrow or spleen. Thus, they discovered that allogeneic transplantation of bone marrow into radiated mice could have extremely successful results if there was no graft rejection¹¹.

The first ever HSCT procedure in humans with leukemia was reported in 1957 by E. Donnall Thomas and although two out of six patients experienced engraftment of the HSCs, none survived after 100 days¹². While all six patients in the study died, knowledge about human leukocyte antigens (HLA) and their role in adaptive immunity was still in its infancy. Nonetheless, many from the scientific community embraced the idea of stem cell therapy to cure the disease of the bone marrow. HLA proteins are found on every cell and uniquely mark them as belonging to the host. When foreign cells are delivered to a patient during transplant, they may not be histocompatible with the host's immune system, increasing the risk of graft rejection.

By the early 1980s, clinical bone marrow transplantation became a much more standard procedure in both autologous (when graft comes from patient) and allogeneic cases. In 1984, a child with acute myelogenous leukemia was cured of both their cancer and SCA using transplant thereby becoming the first patient cured of sickle cell anemia. By the mid-90s, larger studies with more patients were reported. Often the patients chosen were less than 16 years of age with disease complicated by stroke, recurrent ACS and other forms of organ damage. Within two years of the landmark study, 90% were still living after receiving immunosuppressive agents¹³. 18% of patients rejected the graft and others experienced neurologic complications like seizure and hemorrhage. Although the risk of repeat cerebrovascular incident in patients following transplant is reduced, complications like these have resulted in some apprehension to HSCT

Transplant in Sickle Cell Anemia

While HSCT is curative, it is an under-utilized procedure. The barriers to universal use are the issues of safety of transplant preconditioning procedures as well as the availability of (HLA)-matched donors to reduce the risk of graft versus host disease (GVHD). Classically, a patient's bone marrow is depleted of cells with radiation and their immune system is suppressed with chemotherapy prior to the addition of new HSCs. Despite SCA cure rates well

over 80%, transplant related mortality and cost has been a key deterrent for parents, patients, and pharmaceutical companies¹⁴. Myeloablative therapies result in increased treatment related mortality (TRM) in comparison to non-ablative therapies (5.1% v 1.7%) but with decreased rates of graft rejection (15% v 23%) in a series of studies from 1998-2014¹⁵.

Today, physicians endeavor to strike a balance between providing safe, and effective transplant for patients (i.e., low rates of TRM and high levels of engraftment). Moreover, the safety of the procedure is limited by the quality, compatibility and availability of the hematopoietic stem cells for the patient. Less than 20% of sickle cell patients have HLA-matched sibling donor¹⁶. Without any gene-editing, some physicians have sought ways to increase transplantation success from half HLA-matched donors.

Making the State of the Art Safer

From the early days of bone marrow transplantation, increasing age in the patient was a poor prognostic indicator for both success and safety of the procedure. As a result, adults with SCA were much less likely to be cured. In recent years, donor stem cells from unrelated, half HLA-matched donors have been used to cure adults with SCA via improved clinical protocols. In a recent study from the University of Illinois at Chicago, eight adult patients underwent the procedure seven achieved 95% or higher engraftment and one developed GVHD and died of unknown causes¹⁷. The curing of adults with SCA

demonstrates the mileage the medical community has traveled to improve transplant protocols for patients. What may be even more tantalizing is the idea that patients with SCA would have a genetically identical stem cell donor—themselves. The advent of gene-editing and gene therapy has autologous transplant possible for a growing number of patients.

Dealing with Beta-Globin

The approaches below detail the modification of sickle beta-globin expression such that the patient achieves clinical silence of the disease. With the

dawn of CRISPR-Cas9 mediated genome editing in 2012, gene-editing has become more promising than it has ever been. SCA as a monogenetic disorder serves as an excellent disease to attempt gene-correction, and scientists have developed multiple approaches to correcting the genetic defect. Cas9 is a protein that functions as a “molecular scissors” by harnessing an attached guide RNA sequence to bind to a complementary DNA sequence that will be modified¹⁸. Thus, this complex can “recognize” the mutated DNA sequence and



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Harvard University, Chemical Biology

disable it. A corrected form of that DNA can also replace the mutated form. Therefore, the single nucleotide substitution can be corrected in HSCs harvested from the patient via granulocyte colony stimulating factor (G-CSF) mobilization or bone marrow aspiration. The challenging component is an assessment on how many stem cells need to be corrected to produce a desired healthy phenotype in a

patient¹⁹. In the end, the goal is not 100% correction of the genotype, but the generation of a healthy phenotype. People with sickle cell trait carry a copy of mutated beta-globin but by and large live relatively healthy lives. While some scientists aim to edit at the nucleotide level, others wish to modify beta globin expression by lentiviral gene transfer.

Lentiviral gene transfer, a form of gene therapy, allows scientists to add variants of the beta-globin or gamma globin gene to the HSCs of sickle cell patients. This method uses a patient's own HSCs for therapy as well, but instead of using CRISPR to edit, they can add genes using a lentivirus. Gene therapy is becoming a booming industry with different companies engineering their own lentiviral constructs. Bluebird Bio, one of many companies in the increasingly widening gene therapy sector, produced Lenti-Globin BB305. This vector in a preliminary study had been delivered to nine patients and four experienced amelioration of their symptoms of SCA²⁰. In the coming years, more patients will benefit from this new therapy with an expanded trial set to begin in 2022. Other groups have added vectors containing a variant of the gamma globin gene responsible for fetal hemoglobin production with similar results²¹. In addition to unmutated gene addition, other studies have taken a differing approach to a cure with the reactivation of fetal hemoglobin.

Reactivating Fetal Hemoglobin

The SCA mutation of adult hemoglobin, found in the beta-globin gene, is not robustly expressed in the fetus and in the first year of life. Fetal hemoglobin is composed of two alpha and two gamma chains and is resistant to sickling. For decades, scientists have endeavored to harness the anti-sickling capacity of fetal hemoglobin as a therapy for those suffering from the SCA and other beta-hemoglobinopathies²². In 2008, Vijay Sankaran and Stuart Orkin at Harvard Medical School discovered the “molecular switch” that turns on adult globins: BCL11A, a transcription factor which suppresses fetal hemoglobin expression²³. This transcription factor now serves as a therapeutic target. A new clinical trial at Boston Children's Hospital led by David Williams currently aims to deliver lentiviral construct containing a vector

inactivates the BCL11A in erythroid precursors only. BCL11A activity is important in B cell production, which therefore necessitates a targeted inactivation of BCL11A as opposed to a non-specific knockdown. Preliminary data has shown fetal reactivation of at least 80%—well beyond what is needed for a disease-free presentation²⁴.

Towards a Universal Cure

In March 2018, the National Institutes of Health held a special meeting entitled “Accelerating Cures in Hemoglobinopathies.” There, Matthew Porteus of Stanford University posed a challenge to national leaders in SCA “to cure every three year old in the world.” He imagined an age where children with SCA were cured before developing end organ damage. The use of HSCT as a universal cure has not been accomplished in the United States and is even further from implementation in the developing world where the incidence of the disease is the highest. But, as researchers aim to implement key advances in stem cell therapy patients and physicians need to know that these new options now exist. A recent New York Times article shares the stories of patients who are experiencing sickle cell anemia-free living following HSCT with gene-modified autologous HSCs²⁵. These patients are filled with excitement and hope.

In 2019, there are now more successful transplants for every failure and physicians and scientists have developed novel and effective ways to use HSCs to cure SCA. As pre- and post-transplantation regimens improve, the toxicities linked to chemo-radiation will make HSCT more attractive for patients and families.

Scientific advancement can only take place if physicians and patients work together. The responsibility for improved outcomes is in the hands of providers optimizing the procedures as well as patients willing to participate in the much-needed clinical trials. With increased publicity surrounding these new breakthroughs in gene therapy there is hope that when physicians deliver the news of a SCA diagnosis that can also “use the C word” according to NIH Director Francis Collins. What does he mean?

“Cure.”

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